

A M E N D M E N T S

In the Claims:

1. (Previously Presented) A DNA sequence coding for hG-CSF, comprising the nucleotide sequence of SEQ ID NO:1.
2. (Previously Presented) A modified DNA sequence coding for hG-CSF, comprising a nucleotide sequence having at least the following sequence segments, modified with respect to a native sequence coding for hG-CSF, defined by SEQ ID NO:3:
  - a "segment I" (located at the 5' terminal end of the native hG-CSF sequence between nucleotide positions 3 and 194), comprising replacements selected from the group consisting of replacements of *E. coli* rare codons by *E. coli* preference codons, replacements of GC rich regions by AT rich regions, and combinations thereof;
  - a "segment II" (located between nucleotide positions 194 and 309 of the native hG-CSF sequence), comprising replacements of *E. coli* rare codons by *E. coli* preference codons;
  - a "segment III" (located between nucleotide positions 309 and 467 of the native hG-CSF sequence), comprising replacement of a CGG Arg148 codon with a CGT Arg148 codon and replacement of a GGA Gly150 codon with a GGT Gly150 codon; and
  - a "segment IV" (located at the 3' terminal end of the native hG-CSF sequence, between nucleotide positions 467 and 536), comprising replacements of *E. coli* rare codons by *E. coli* preference codons.
3. (Previously Presented) A DNA sequence according to claim 2, which encodes a biologically active G-CSF.
4. (Currently Amended) A DNA sequence according to claim 3 1, wherein the sequence provides an expression level of G-CSF, of the total proteins after expression, of at least 50% in an expression system, as quantified by staining protein bands after separation by SDS-PAGE.
5. (Previously Presented) A DNA sequence according to claim 2, further comprising a 5'-untranslated region of the native hG-CSF sequence.

6. (Previously Presented) An expression plasmid, wherein the plasmid comprises a DNA sequence according to claim 1 and a plasmid vector.
7. (Previously Presented) An expression plasmid, wherein the plasmid comprises a DNA sequence according to claim 2 and a plasmid vector.
8. (Previously presented) An expression plasmid according to claim 6, wherein the plasmid vector comprises a T7 promoter sequence.
9. (Previously presented) An expression plasmid according to claim 6, wherein the plasmid vector is selected from the group of pET vectors.
10. (Previously Presented) An expression plasmid according to claim 6, wherein the plasmid vector further comprises a resistance gene selected from the group consisting of an ampicillin resistance gene and a kanamycin resistance gene.
11. (Previously Presented) An expression system for the expression of a DNA sequence coding for hG-CSF wherein the sequence comprises the nucleotide sequence of SEQ ID NO:1, and wherein the system comprises the expression plasmid according to claim 6 and a production strain of *E. coli*.
12. (Canceled)
13. (Previously Presented) An expression system according to claim 11, wherein the production strain is *E. coli* BL21 (DE3).
14. (Previously Presented) An expression system according to claim 13, wherein the expression system is substantially free of an antibiotic.

15. (Currently Amended) A process for construction of a modified DNA sequence according to claim 2, wherein the process comprises:
  - (i) applying methods selected from the group consisting of *de novo* oligonucleotide synthesis, site-directed mutagenesis, oligonucleotide-directed mutagenesis, and combinations thereof, in order to provide a modified DNA sequence coding for hG-CSF, which is modified relative to the native sequence coding for hG-CSF[[],] by modifications selected from the group consisting of:  
the replacement of at least some *E. coli* rare codons with *E. coli* preference codons,  
the replacement of at least some GC rich regions with AT rich regions, and  
combinations thereof; and
  - (ii) maintaining at least a portion of the native sequence coding for hG-CSF unchanged.
16. (Previously Presented) A process for construction of a DNA sequence according to claim 15, wherein the modified DNA sequence further comprises a 5'-untranslated region of the native hG-CSF gene, wherein the process does not involve changes in the 5'-untranslated region in one or more of the following regions: translation initiation region, ribosome binding site and the region between the start codon and the ribosome binding site.
17. (Previously Presented) A process for construction of a DNA sequence according to claim 15, wherein maintaining at least a portion of the native sequence coding for hG-CSF further comprises providing a completely unchanged sequence, relative to the native sequence coding for hG-CSF, in segment III of at least 99 nucleotides in length.
18. (Previously Presented) A process for construction of a DNA sequence according to claim 15, further comprising inserting said DNA sequence into a plasmid vector which comprises a T7 promoter sequence.
19. (Previously Presented) A process for construction of a DNA sequence according to claim 15, wherein the DNA sequence provides protein expression level in *E. coli*, of at least 50% of the total proteins expressed, as quantified by staining protein bands after separation by SDS-PAGE.

20. (Previously Presented) A process for the expression of hG-CSF, comprising expressing in *E. coli* a DNA sequence according to the expression plasmid of claim 6.
21. (Previously Presented) A process for expression of hG-CSF according to claim 20, wherein IPTG is used for induction at a concentration in the range of about 0.1 mM to about 1 mM.
22. (Previously Presented) A process according to claim 20, which comprises a fermentation step performed at a temperature of about 20°C to 30°C.
23. (Canceled)
24. (Withdrawn) A process for the manufacture of a pharmaceutical composition comprising hG-CSF or biologically active G-CSF, wherein said process comprises:
  - (a) carrying out a process according to claim 20,
  - (b) isolating and/or purifying the hG-CSF or biologically active G-CSF obtained by step (a), and
  - (c) mixing the isolated and/or purified hG-CSF or biologically active G-CSF with a pharmaceutically acceptable carrier or auxiliary substance.
25. (Previously Presented) A process according to claim 20, wherein the hG-CSF is in inclusion bodies.
26. (Previously Presented) A DNA sequence according to claim 3, wherein the biologically active G-CSF further comprises G-CSF in inclusion bodies.